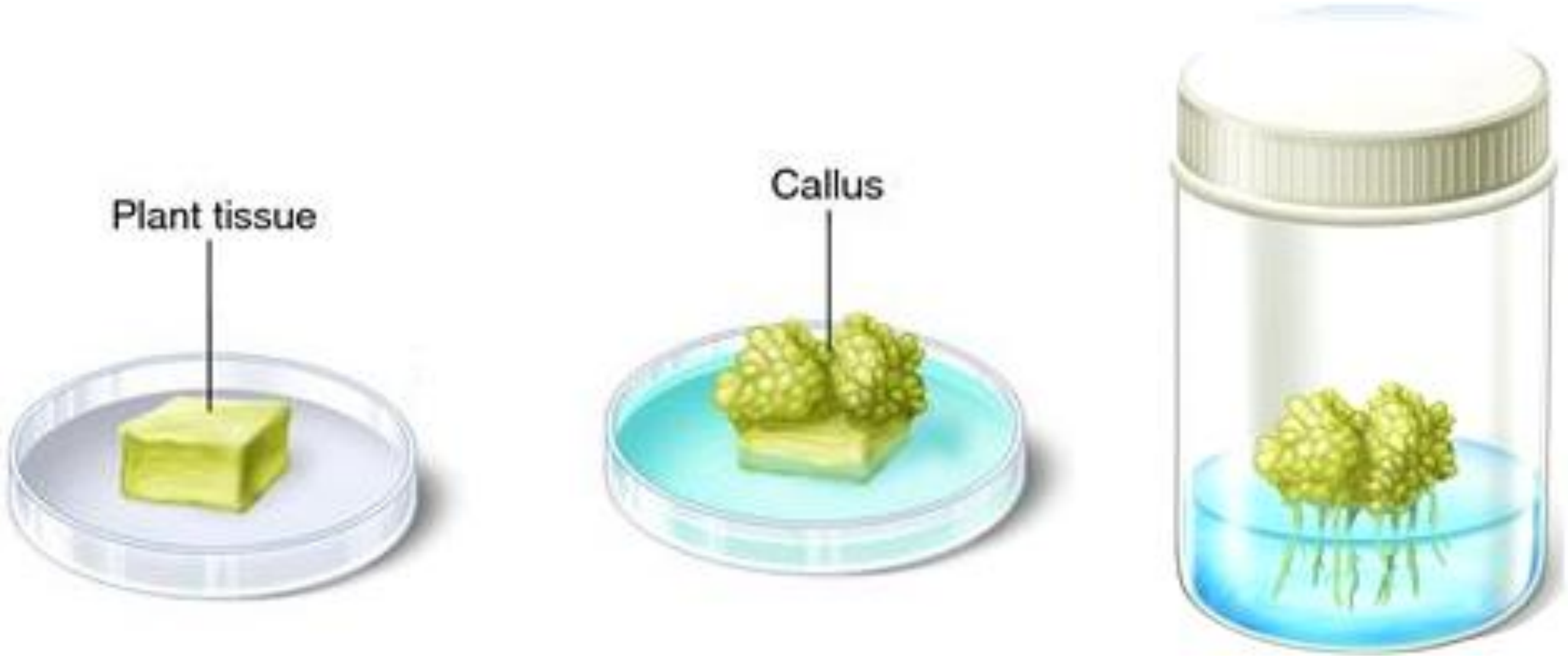
Plant Cell & Tissue Culture

Prof. Dr. Omar Sabry

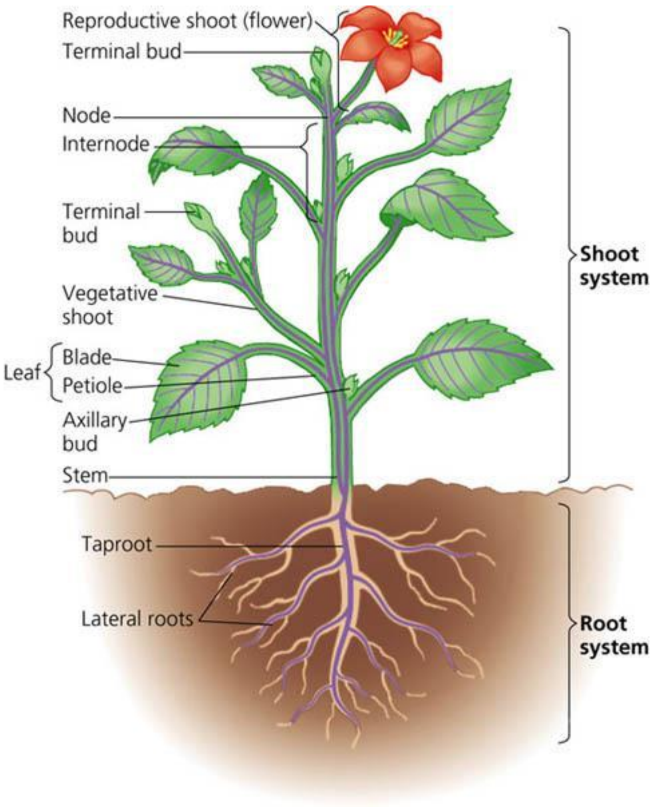
Culture Vessels

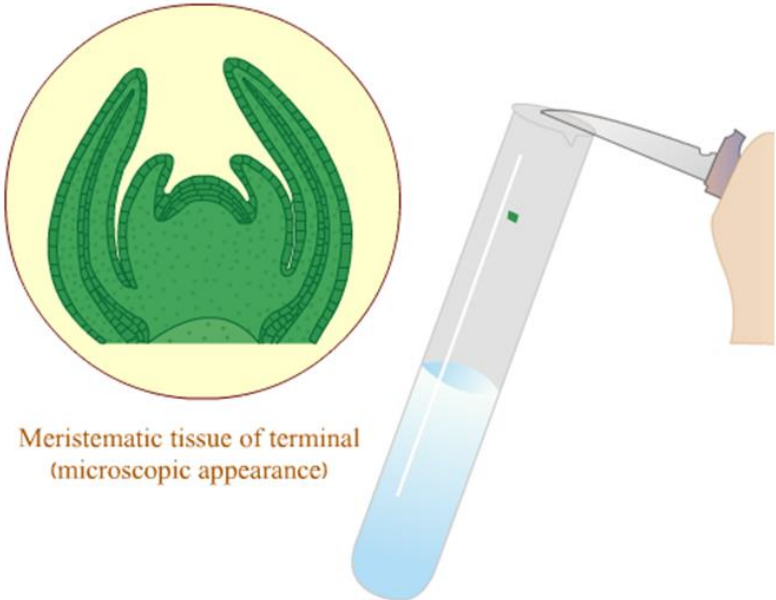
- ☐ **Borosilicate or Pyrex glassware should be used.**
- ☐ **Soda glass toxic to tissues with repeated use.**



Sources of tissue explants

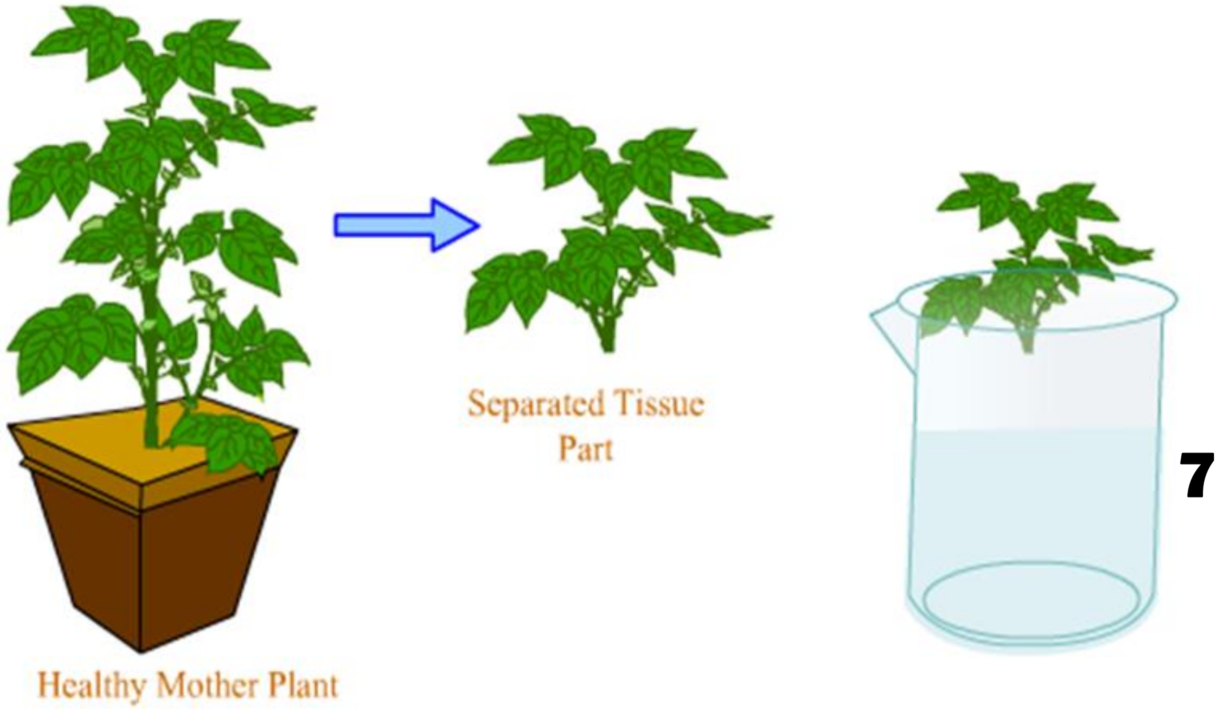
- Organ with meri-stimatic cells Pith, root tips, shoot tips, embryos, anthers and young leaves.





Sterilization of plant material

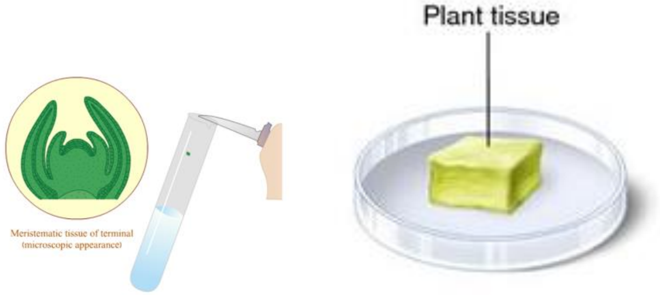
- Tissues must be surface sterilized before planting on the nutrient medium.



70% Ethanol

Sterilizing agents used in plant sterilization

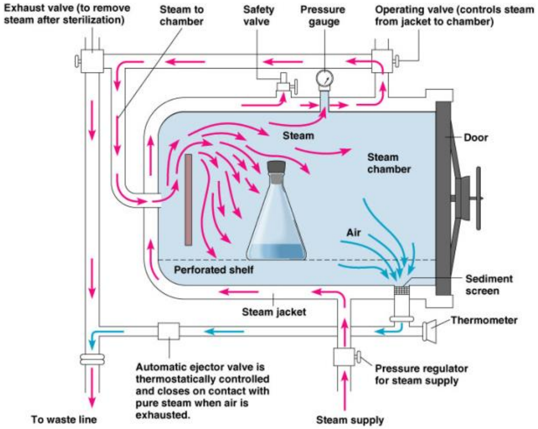
- **70% ethyl alcohol then Sodium hypochlorite 1%**
- **(No methanol)**
- **Sodium hypochlorite 25%**



- **After disinfection, carefully trim by cutting away all bleached and dead tissue .**
- **Rectangular or cylindrical pieces approximately 5 mm in diameter excised transferred to agar plates containing nutrient medium.**

Sterilization of glass ware, plastic ware and other instruments

- Autoclaving or dry heating in an oven at 160 -180°C for 3hr.

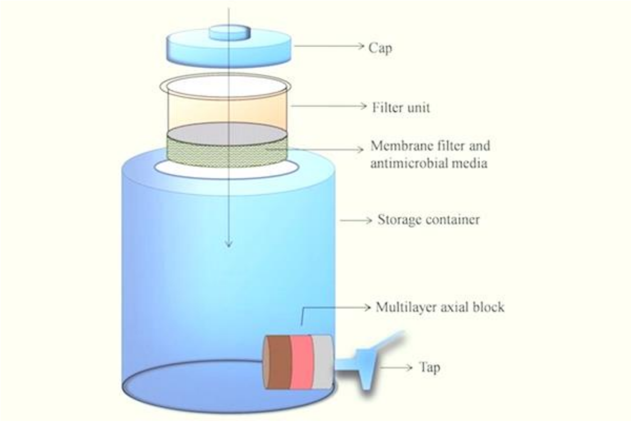

Exhaust valve (to remove steam after sterilization)
Steam to chamber
Safety valve
Pressure gauge
Operating valve (controls steam from jacket to chamber)
Steam
Door
Steam chamber
Air
Perforated shelf
Sediment screen
Steam jacket
Thermometer
Automatic ejector valve is thermostatically controlled and closes on contact with pure steam when air is exhausted.
Pressure regulator for steam supply
To waste line
Steam supply

- Plastic ware can be heat sterilized by autoclaving at 121 °C.

- Forceps, scalpels, needles and spatulae sterilized by 70% or 95 % ethanol followed by flaming and cooling.

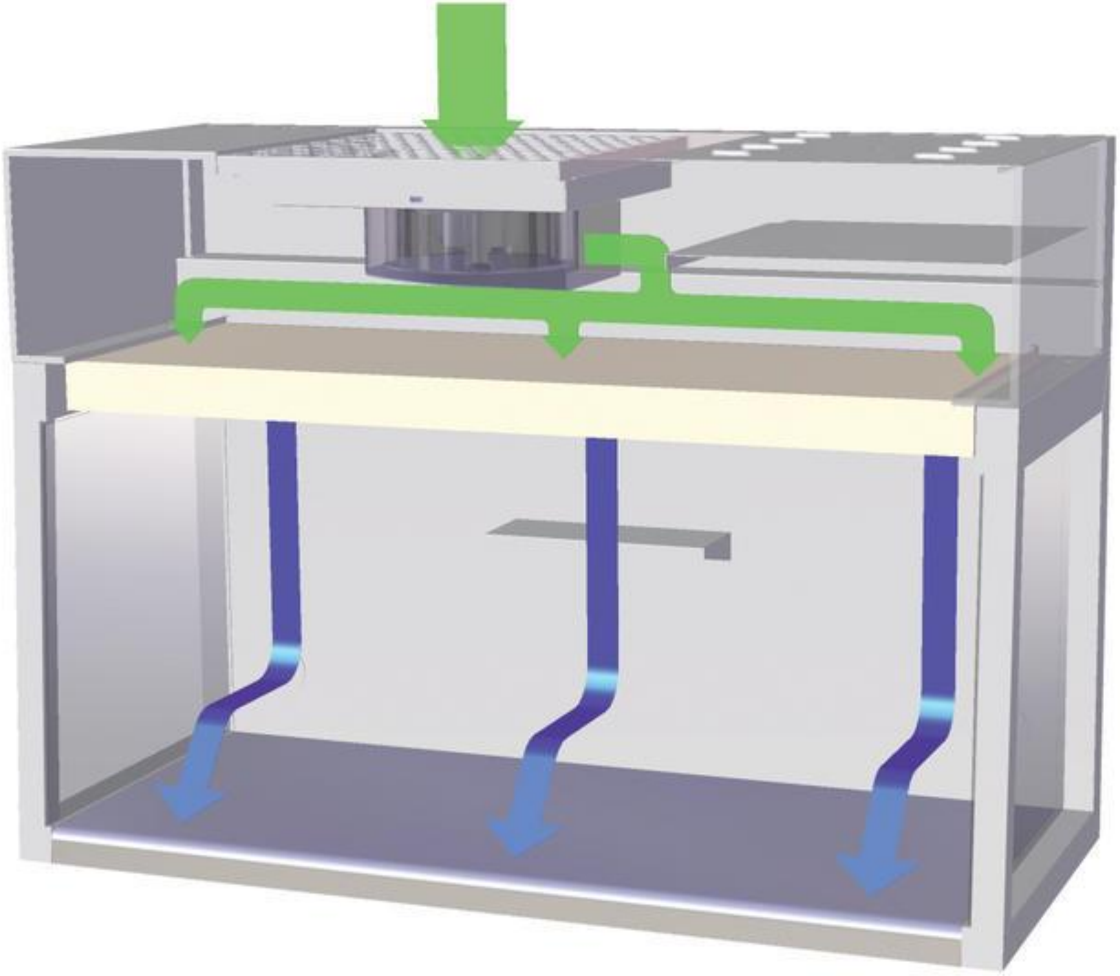
Sterilization of Media

- Some growth factors are thermolabile (No autoclaving).
- Medium minus the heat labile compound autoclaved in a flask kept in the sterilized hood to cool down.
- Solution of the thermolabile compound is sterilized by Micropore membrane filter.



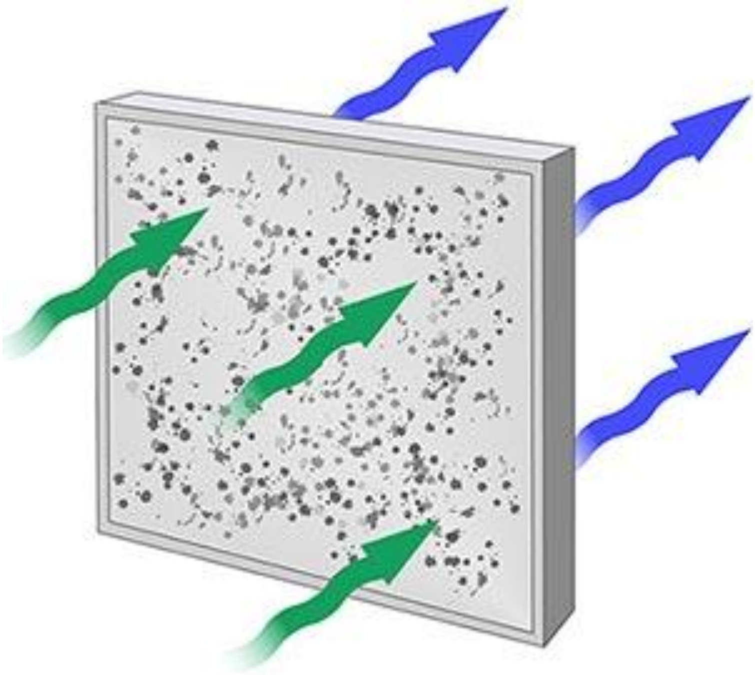
Sterilization of Transfer Area

- **Laminar airflow cabinets**



Sterilization of Air

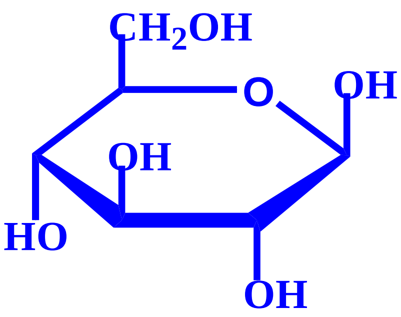
- **High efficiency particulate air (HEPA) filter**

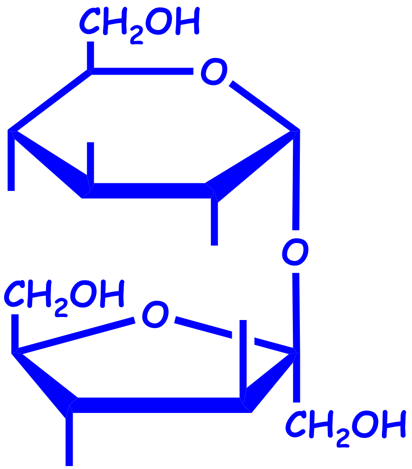


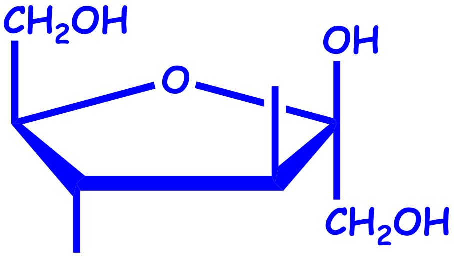
Media Constituents

❑ **Inorganic Nutrients**

- **Macro - or major elements (6): e.g. Nitrogen – Calcium.**
- **Micro - or minor elements (6): e.g. Iron - Cu**







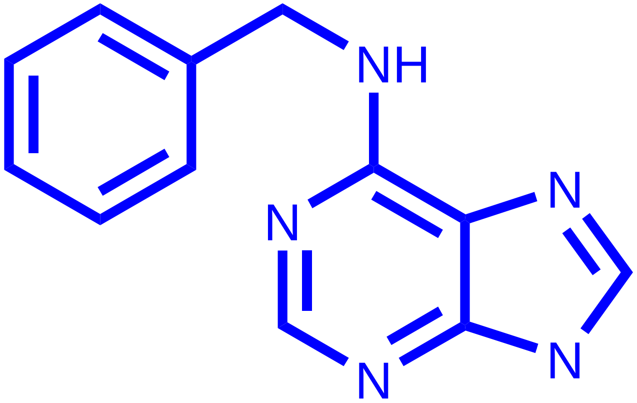
❑ **Organic Nutrients**

- **Nitrogenous substances e.g. Vitamins. Thiamine - Pyrodoxol**
- **Carbon Source e.g. Sucrose 2-5%, glucose and fructose**

Growth Hormones

CYTOKININS

- ☐ **BAP (benzyl amino purine)**



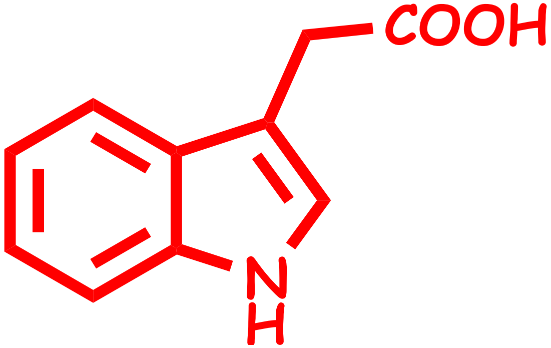
- ☐ **Shoots emerge, Plant rooted with auxin, transferred to potting soil for further growth in the greenhouse as normal plants.**

- ☐ **Equal concentrations gives unorganized fashion**

Growth Hormones

AUXINS

- IAA (Indole acetic acid)



- Excess of auxins promotes growth of roots

- Excess of cytokinins yield shoots.

Agar

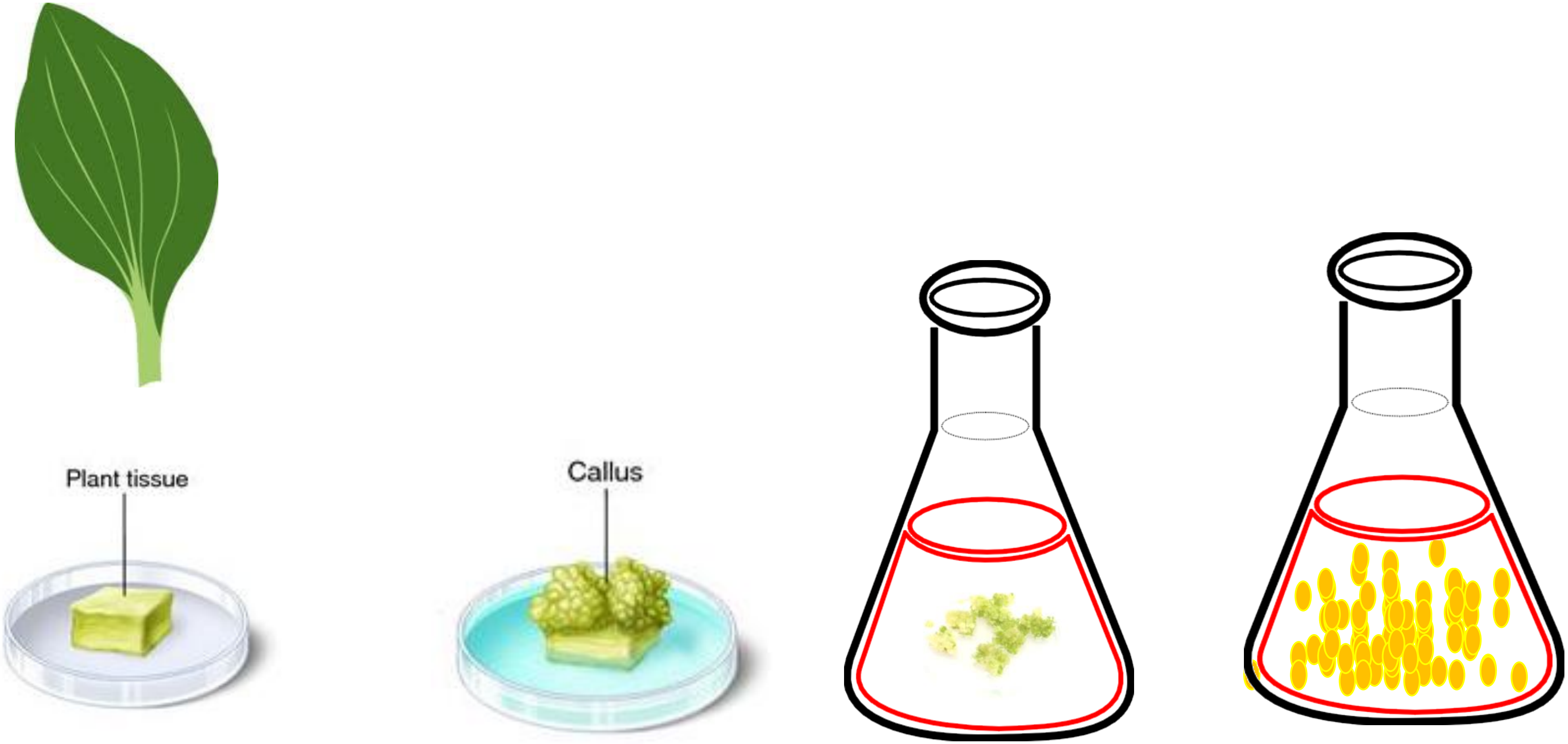
- ☐ Generally used at a concentration of 0.8 - 1.0%.

- ☐ Higher concentration the medium becomes hard not allow the diffusion of nutrients into the tissues.

- ☐ Not an essential components of the nutrient medium.

Cell Suspension Culture

- Single cells and aggregates can be grown as suspension cultures in liquid medium.



Cell Suspension Culture

- **Regularly aerated either by bubbling sterile air, or gentle agitation.**

- **Liquid medium proves superior to agarified medium.**

- **pH of the medium 5.0 - 6.0 before sterilization.**

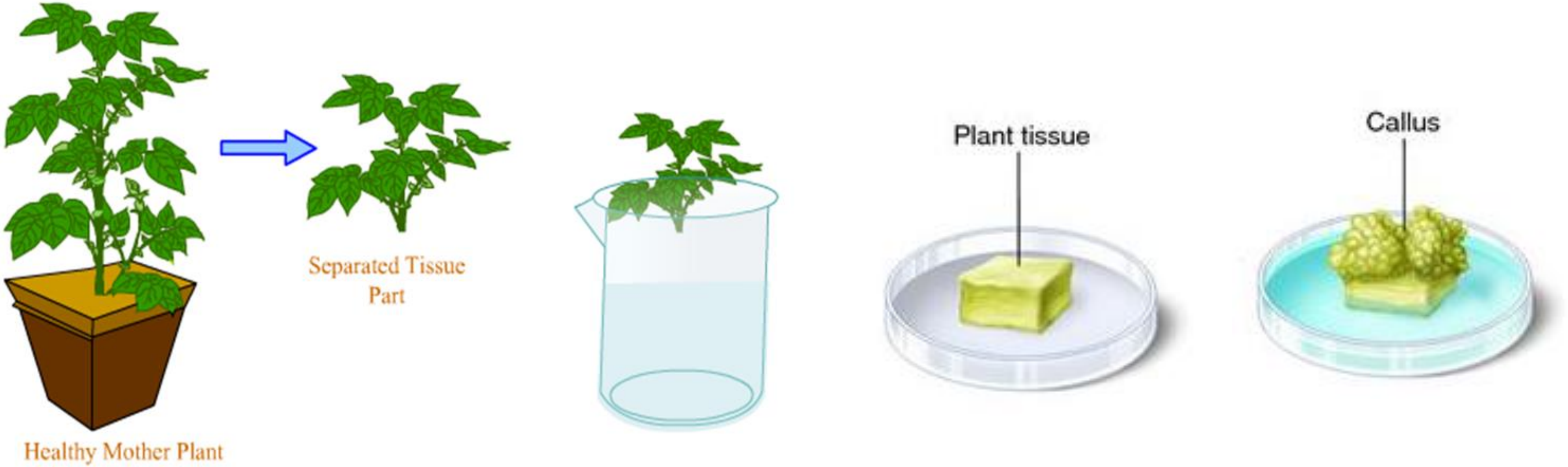
Media Selection

- Start with known basal medium.
- Minor qualitative and quantitative changes, through series of experiments a new medium may be evolved.
- Simplest method commercially available dry powdered media, containing inorganic salts, vitamins and amino acids.

Stages of Tissue culturing

1- PREPARATION OF THE EXPLANT

- Piece of stem, leaf, or root is disinfected with alcohol or sodium hypochlorite solution and cultured in a specific culture medium.



Stages of Tissue Culturing

2- MULTIPLICATION

- ☐ Number of cells increases.
- ☐ Cells cultured from a callus in the presence of low concentration of auxin and cytokinin.
- ☐ Calli placed in liquid medium and grown in suspension or placed on a solid substrate to be cultured.

Stages of Tissue Culturing

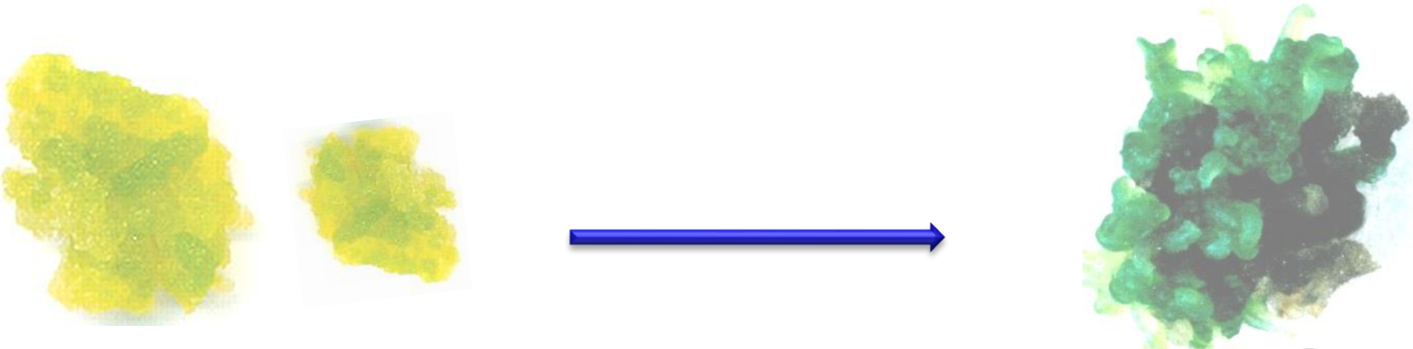
- **Callus cultures subdivided and re-cultured .**
- **Exposed to growth hormones, Calli form shoots and roots.**
- **Callus formation and maintenance must be taken in consideration because when cultured for several weeks any callus show signs of aging.**

Stages of Tissue Culturing

3. PRE-TRANSPLANT STAGE

- Allows the formation of roots, shoot development, maturation, and development.
- Cytokinins are reduced or eliminated and auxins are added to promote root development.

Factors inducing aging



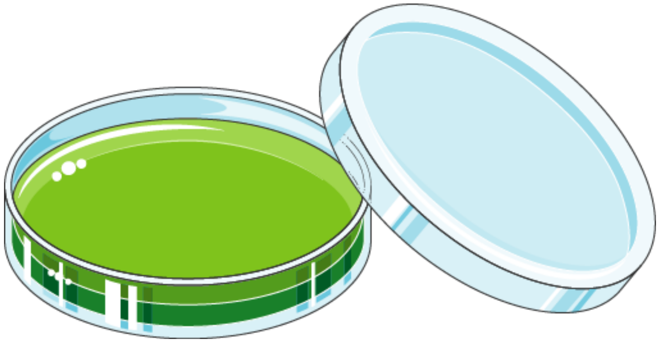
- ☐ **Exhaustion of the nutrients.**
- ☐ **Inhibition of nutrient diffusion.**
- ☐ **Accumulation of toxic metabolites {Add activated charcoal to avoid this factor}.**
- ☐ **Periodically transfer to fresh medium (sub-culture) at intervals of 4-6 weeks.**

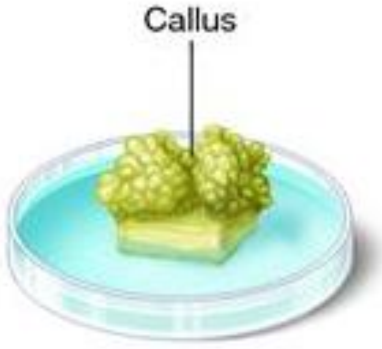
Preparation of Cell suspension Cultures

- Sterilized plant organs on a nutrient medium containing suitable hormones (auxins and cytokinins).

- The explant exhibits callusing.

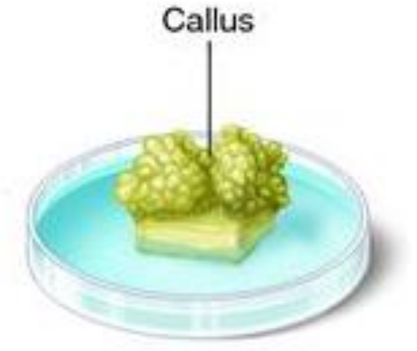
- Callus separated from the parent explant transferred to fresh medium.



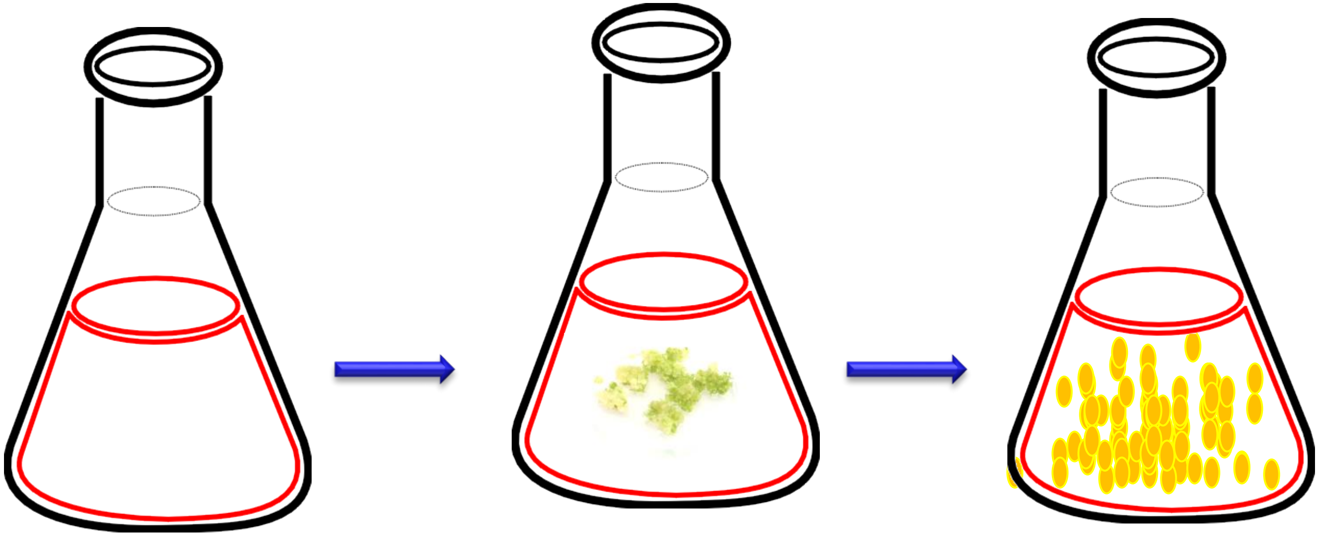


Preparation of Cell Suspension Cultures

- Repeated sub-culture on the agar improves friability desirable for raising a fine cell suspension in liquid medium.



- On transfer to liquid medium gave fine suspension.

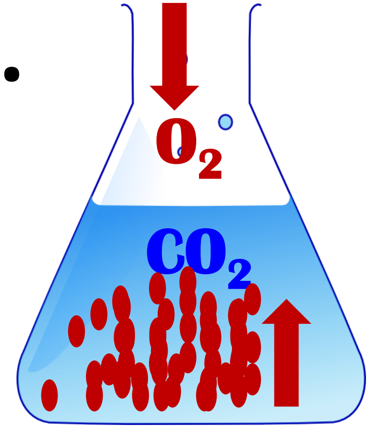


Agitation Functions

- **Breaking clumps into single cells.**

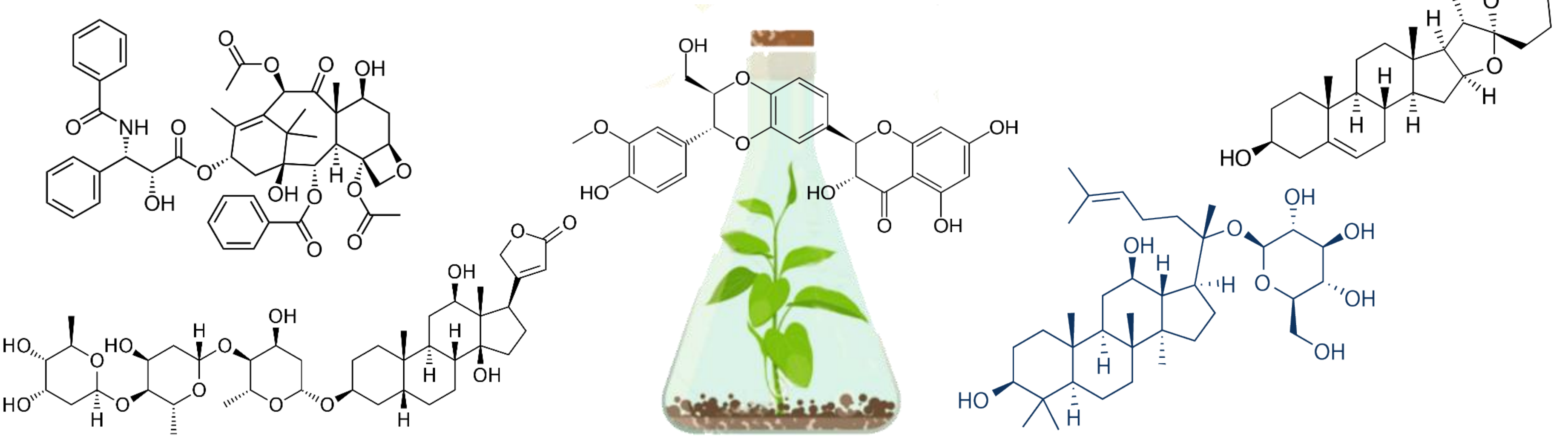
- **Uniform distribution of cells in the medium.**

- **Gaseous exchange.**



Practical Exam {10 Marks}

Presentation



☐ **Pharmacologically Bioactive Compound**

☐ **Importance and uses**

☐ **Elicitation and In-vitro production**

☐ **Each group 5 students**

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Thank You